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(54) Title: PLANT STRESS REGULATED GENES

(57) Abstract: The present invention relates to a method to isolate plant genes or gene fragments that are regulated by stress, preferably oxidative stress in plants. The method comprises isolation of plant material, adaptation of the plant material to stress, differential expression of genes or gene fragments in adapted and non-adapted plant material, and isolation of the differential expressed genes or gene fragments. The invention further relates to the genes or gene fragments that can be obtained by this method and to the use of these genes or gene fragments to modulate plant stress tolerance.

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PLANT STRESS REGULATED GENES

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The present invention relates to a method to isolate plant genes or gene fragments that are regulated by stress, preferably oxidative stress in plants. The method comprises isolation of plant material, adaptation of the plant material to stress, differential expression of genes or gene fragments in adapted and non-adapted plant material, and isolation of the differentially expressed genes or gene fragments. The invention further relates to the genes or gene fragments that can be obtained by this method and to the use of these genes or gene fragments to modulate plant stress tolerance.

Plant molecular responses to environmental stresses are generally very complex and often result in alteration of gene and protein expression as well as in changes in metabolic profiles (Sandermann *et al.*, 1998; Jansen *et al.*, 1998; Somssich and Hahlbrock, 1998; Bartels *et al.*, 1996). At least some of those stress responses are required for enhanced stress tolerance as the moderate doses of many stresses increase plant resistance to deleterious stress conditions. For example, raising the temperatures slowly to high, non-lethal temperatures allows plants to tolerate temperatures that are normally lethal, a phenomenon referred to as acclimation (Vierling, 1991). Similarly, exposing maize plants to 14°C acclimates them to lower temperatures that would normally cause chilling injuries (Prasad *et al.* 1994). Also pathogen infection often leads to resistance against subsequent challenges by the same or even unrelated pathogen (reviewed in Sticher *et al.*, 1997). This phenomenon of induced stress tolerance is not restricted to the same kind of the stress and cross-tolerance induced by different kind of stresses has been reported (Örvar *et al.*, 1997; Orzech and Burke, 1988; Keller and Steffen, 1995; Cloutier and Andrews, 1984).

Much of the damage due to environmental constrains has been attributed to the excess production of active oxygen species (AOS), so called oxidative stress (reviewed in Inzé and Van Montagu, 1995). Plant cells acclimated to heat and cold as well as plants expressing systemic acquired resistance to pathogens show also enhanced capacity to tolerate oxidative stress (Banzet *et al.*, 1998, Seppänen *et al.*, 1998, Strobel and Kuc, 1995). This suggests that induced tolerance to oxidative stress is part of the adaptation mechanism to environmental stresses and likely contributes to

the observed phenomenon of cross-tolerance. However, little is known in plants about molecular mechanisms underlying induced tolerance to oxidative stress.

In contrast, adaptive responses to various oxidants have been extensively studied in bacteria and yeast. In both *E. coli* and *S. cerevisiae*, adaptation to oxidative stress is an active process requiring *de novo* protein synthesis (Davies *et al.*, 1995, Storz *et al.*, 1990). At least 80 proteins are induced by adaptive amounts of oxidants in *E. coli*; 40 of them belong to H₂O₂ stimulon and 40 to O₂ stimulon. Among the induced enzymes are antioxidant enzymes, DNA repair enzyme, heat shock proteins and glucose-6-phosphate dehydrogenase implicated in energy homeostasis (reviewed in Demple, 1991).

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Yeast, similarly to bacteria, possess at least two distinct but overlapping adaptive stress responses to oxidants: one induced by H_2O_2 and the other by $O_2^{\bullet-}$ generating compounds (Jamieson, 1992). The H_2O_2 stimulon has been analysed by comparative two-dimensional gel analysis of total cell proteins isolated after treatment with low doses of H_2O_2 (Godon *et al.* 1998). Such a treatment resulted in synthesis of at least 115 proteins and repression of 52 proteins. 70% of those proteins have been identified and classified into cellular processes such as antioxidant defences, heat shock responses and chaperone activities, protein turnover, sulphur, amino acids, purine, and carbohydrate metabolism. Notably, carbohydrate metabolism was redirected to the regeneration of NADPH, which provides reducing power necessary for the detoxification of active oxygen species.

In plants, tolerance to oxidative stress has been previously associated with enhanced activity of antioxidant enzymes and levels of antioxidant metabolites (reviewed in Inzé and Van Montagu, 1995). In addition, Banzet *et al.* (1998) have demonstrated that other stress proteins are likely implicated in acquisition of oxidative stress tolerance by plant cells, similarly as in lower organisms. Expression of small heat shock proteins correlated with adaptation of tomato cells to oxidative stress and additionally, heat shock pre-treatment was able to enhance resistance of those cells to oxidative stress. However, no comparative genome-wide characterisation of induced adaptive responses to oxidative stress has been undertaken in plants.

A genomic approach was used to study the adaptive responses to oxidative stress in leaf tissue of *Nicotiana tabacum*. The redox-cycling compound methyl viclogen (MV; paraquat) was used to induce an adaptive response to oxidative stress, as AOS signalling may be important during the defence against both biotic and abiotic stresses

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in plants (Levine et al., 1994, Prasad et al., 1994, Banzet et al., 1998, Chamnongpol et al., 1998, Alvarez et al., 1998, Karpinski, 1999). Surprisingly, we found that adaptive amounts of MV enhance the tolerance of tobacco leaf tissues to oxidative stress imposed by toxic levels of the same oxidant. Moreover, adaptation to oxidative stress is associated with induction/repression of approximately 170 genes and partial characterisation of induced genes shows that they are implicated in distinct cellular processes. Several of these defence responses induced by adaptive amounts of oxidants have so far never been associated with the antioxidant response.

It is a first aspect of the invention to provide a method to isolate stress regulated genes or gene fragments, said method comprising

(a) isolating plant material

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- (b) inducing stress adaptation in said plant material
- (c) checking differential expression between stress adapted and non-adapted plant material
- 15 (d) isolating differentially expressed genes or gene fragments.

Plant material can be any plant material, such as parts of, or complete, roots, stems or leaves. Plant material may include more than one plant tissue, up to a complete plant. Preferably, said plant is a tobacco plant. Even more preferable, said plant material is leaf material.

Induction of stress adaptation is preferentially carried out by applying sub-lethal stress to said plant material. Stress can be any biotic or abiotic stress, such as fungal or bacterial infection, heat or cold treatment, or oxidative stress. Preferably, said stress is oxidative stress. More preferably, said oxidative stress is applied by putting said plant material in a solution comprising an adequate amount of methyl viologen (methyl viologen pre-treatment). Alternatively, the sub-lethal stress phase may be followed by a period of stronger stress. Said stronger stress may even result in significant cell damage when applied to unadapted plant material.

Differential expression includes induction as well as repression. Checking differential expression can be done with all the differential expression or differential display techniques know to the person skilled in the art, such as, but not limited too, messenger substraction, filter hybridization or micro-array techniques.

Isolation of the differentially expressed genes may be direct or indirect, i.e. by direct isolation of the differentially expressed nucleic acid such as mRNA or cDNA, or by isolation the genes from a library, on the base of the results identifying the gene, such

as filter hybridisation or micro-array. Preferably, the differentially expressed genes or gene fragments are isolated using PCR-based techniques.

A further aspect of the invention is a gene, or gene fragment, obtained by the method according to the invention. A preferred embodiment is a gene or gene fragment, comprising a sequence selected from any of the sequences from SEQ ID N° 1 to SEQ ID N° 167.

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Clone names of these sequences, their expression pattern and an indication of the function by homology search is summarized in Table 1.

An even more preferred embodiment is a gene, encoding a protein comprising, preferably essentially consisting, more preferably consisting of SEQ ID N° 169. Preferably, said gene comprises SEQ ID N° 168, more preferably said gene is essentially consisting of SEQ ID N° 168, even more preferably said gene is consisting of SEQ ID N° 168.

Still another aspect of the invention is the use of a gene or a gene fragment according to the invention, or a gene that is at least 60% identical, preferably 80% identical, more preferably 90% identical to said gene or gene fragment according to the invention, or a gene fragment from a gene that is at least 60% identical, preferably 80% identical, more preferably 90% identical to said gene or gene fragment according to the invention to modulate plant stress tolerance. A preferred embodiment is the use of a gene or gene fragment, comprising SEQ ID N° 168, preferably essentially consisting of SEQ ID N° 168, more preferably consisting of SEQ ID N° 168. Preferably, said stress is oxidative stress. Preferably, said plant is tobacco.

A special embodiment is the use of a gene fragment according to the invention, whereby said gene fragment is a promoter. Although the gene fragments isolated by the differential expression procedure may be coding sequences that do not comprise the promoter of the gene, it is obvious for the person skilled in the art to isolate the promoter of a gene when the coding sequence is known. As a non-limiting example, the coding sequence can be used as a probe against a genomic library, whereby the positive scoring clones are subcloned, and the positive subclone is sequenced. On the base of the sequence, the promoter part and the coding part, including the intron – exon boundaries can be predicted using computer software, such as Genemark, Genscan or Grail. Alternatively, the full-length messenger RNA can be isolated, and on the base of its sequence, the start of transcription can be defined, and the promoter can be localized.

Another aspect of the invention is a vector comprising a gene or a gene fragment according to the invention. Said vector may be any vector suitable for eucaryotic cells, as is known to the person skilled in the art, and include but are not limited to self replicating vectors, integrative vectors and virus-based vectors. Preferably, said vector is a plant transformation vector and said eucaryotic cell is a plant cell.

Still another aspect of the invention is a method to modulate stress tolerance in a plant cell or plant, comprising the introduction of the vector according to the invention in said plant cell or plant. Introduction of the vector in the plant cell or plant can be realized by any suitable technique known to the person skilled in the art and includes, but is not limited to transformation techniques such as electroporation, particle bombardment or *Agrobacterium*-mediated transformation, floral dip transformation or sexual techniques such as crossing.

A further aspect of the invention is a plant cell or plant, comprising a vector according to the invention. Preferably, said plant cell or plant is a tobacco plant cell or plant.

DEFINITIONS

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Plant material can be any plant tissue such as root, stem or leaf. It may be a part of the plant, such as a disc excised from the leaf, up to the intact plant.

Adaptation as used here means the application of a stress to the plant for a certain time, whereby the time and/or the level of stress are controlled in such a way that the stress applied over the time used is sub-lethal. Sub-lethal stress as used here refers to stress that may result in a specific gene expression pattern, but is not leading to cell damage. Detrimental tissue damage can be evaluated by several methods known to the person skilled in the art, but is preferably evaluated by measuring an increase in conductivity as described in the examples. An increase in conductivity in the stress situation, compared with a non-stressed reference situation by less than a factor 5, preferably less than a factor 2, as measured after 42 hours of stress application is considered as non significant.

The term *gene* as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. The term includes double- and single-stranded DNA and RNA. It also includes know types of modifications, for example methylation, "caps" substitution of one or more of the naturally occurring nucleotides with an analogue. It

includes, but is not limited to, the coding sequence. It does include the regulatory sequences such as the promoter and terminator sequences.

Gene fragment may be any gene fragment of at least 40 contiguous nucleotides, preferably 60 nucleotides, more preferably 100 nucleotides, either coding or non-coding. A special case of gene fragment is the promoter of the gene.

Modulation of stress tolerance as used here comprises both the increase of stress tolerance, as well as the decrease of stress tolerance, independent of the level of decrease or increase.

% identical is the percentage identity as measured by a TBLASTN search (Altschull et al., 1997).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Effect of different concentrations of methyl viologen on leaf discs damage.

Three leaf discs were floated on solution with assigned methyl viologen concentrations for indicated time periods. Ion leakage was measured as conductivity of the medium at indicated time intervals. Experiment was done in duplicate and presented value is the average of both measurements. The conductivity of the solution was subtracted from the measured values.

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Figure 2. Effect of MV pre-treatment on leaf discs tolerance to 1μM methyl viologen. Leaf discs that were pre-treated for 17 hours with water (grey bars) or 0.1μM methyl viologen (black bars) were exposed to 1μM solution of methyl viologen. Ion leakage was measured as conductivity of the medium in the course of the treatment at regular intervals. The conductivity of the solution was subtracted from measured values. Presented values are average values of nine independent experiments.

- **Figure 3.** Expression of GPx and SodCc during the treatment with $1\mu M$ methyl viologen.
- Leaf discs pre-treated with water (0) or 0.1μM MV (0.1) for 17 hours were exposed to 1μM methyl viologen and expression of a glutathione peroxidase gene (*GPx*) and a gene encoding cytosolic CuZnSOD (*SODCc*) was analysed. Total RNA (5 μg) was extracted from 6 leaf discs sampled in two independent experiments at indicated times and subjected to Northern analysis. The same membrane was used for hybridisation

with both genes. Hybridisation of the constitutive actin gene was used as a loading control (bottom panel).

Figure 4. Expression of genes isolated by differential display during the pre-treatment with 0.1µM methyl viologen and the treatment with 1µM methyl viologen.

Total RNA was extracted from 9 leaf discs sampled at indicated times before (c) and during the pre-treatment with $0.1\mu M$ MV (0.1) or water (0), and after exposure of pre-treated samples to $1\mu M$ MV. Blots with $15\mu g$ total RNA each were prepared in quadruplicates and checked for equal loading by methylene blue staining. Each membrane was reused several times.

Figure 5. Resistance to MV of *A. thaliana* transformed with WRKY11 fused to the VP16 activation domain, under control of the 35S promoter. (A) control plate without MV; (B) test plate with 2μM MV. WV9 and WV4: transformed lines, C24: untransformed control.

EXAMPLES

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Materials and methods to the examples

Plant Material and Cultivation Conditions.

Nicotiana tabacum cv. Petit Havana SR1 plants were grown in a controlled environment chamber (Weiss technik, Lindenstruth, Germany) under 100 μ mol/m²/s light intensity (photosynthetically active radiation), 16h light/ 8h dark regime, relative humidity of 70% and constant temperature of 24°C. The most expanded leaves (11-12 cm long x 7-8 cm wide) from 5 week old plants were used for experiments with methyl viologen.

Methyl Viologen Treatment.

Leaf discs (1cm in diameter) were punched with a cork-bore from the intervenal part of the leaf. Three leaf discs, each originated from different plants, were floated with the abaxial side up on 12 ml of methyl viologen solution in nanopure water or water solely in the case of control. Treatments were performed in controlled environment chambers, under the same conditions as for growth, except otherwise indicated. Leaf

discs for RNA extraction were drained on paper, rapidly frozen in liquid nitrogen and stored at -70°C. Ion leakage from the leaf discs was measured as conductivity of the solution using a conductivity meter (Consort, Turnhout, Belgium).

5 RNA Extraction and Northern Analysis

Total RNA was extracted from frozen leaf discs using TRIzol™ Reagent (Life Technologies, Paisley, UK) according to the manufacturer's instructions. RNA samples were treated prior to electrophoresis and resolved on 1% agarose gel as described by Shaul et al. (1996). The RNA was blotted on nylon membrane (Hybond-N, Amersham International plc., Buckinghamshire, UK or Qiabrane, Qiagen GmbH, Hilden, Germany) by capillary blotting (Maniatis et al., 1982). RNA was fixed to the membrane by crosslinking at 150mJ/cm². To check the quality of RNA prior to hybridisation, membranes were incubated for 15 minutes in 5% acetic acid and stained for 5 minutes in 0.04% methylene blue in 0.5 M sodium acetate (pH 5.2), and rinsed with water. After the staining and quality check, membranes were destained in 0.1 x SSC (Maniatis et al., 1982) containing 0.5%SDS (w/v). Membranes were hybridised at 65°C in 50% formamide, 5x SSC, 0.5% SDS and 10% dextran sulphate. ³²P-labelled RNA probes corresponding to the cDNA fragments of GPx (Criqui et al., 1992), SodCc(pSOD3-5'fragment; Tsang et al., 1991), SodB (pSOD2-5'fragment; Tsang et al., 1991), Cat1 (pCat1A; Willekens et al., 1994) and N. tabacum actin (pRVA12; AventisCropScience, Belgium) were generated by the Riboprobe® System (Promega Corp., Madison, WI, USA). RNA probes corresponding to cDNA fragments isolated by differential display and cloned into pGEM®-T vector (Promega Corp., Madison, WI, USA) were generated according to the same protocol. Membranes were washed at 65°C for 15 minutes each in 3 x SSC (Maniatis et al., 1982), 1 x SSC and 0.1 x SSC (stringent washing) containing 0.5% SDS (w/v). Membranes were exposed to the Storage Phosphor Screen and scanned with the Phosphorlmager 445 SI (Molecular Dynamics Inc., Sunnyvale, CA, USA). Membranes were reused after stripping of the probe in 0.1 x SSC at 85°C. Removal of the probe was checked by autoradiography.

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Differential display

Total RNA was treated with DNasel prior to RT-PCR according to the manufacturer's instruction (Life Technologies, Paisley, UK). Alternatively, up to 20 μg of total RNA was incubated with 5U DNasel, 18U Recombinant Ribonuclease Inhibitor (Promega Corp.,

Madison, WI, USA), 1mM DTT in 80µl of 10mM Tris-Cl, pH8,3, 50mMKCl and 1,5mM MgCl₂ for 30 minutes at 37°C. RNA was extracted with phenol/CHCl₃ (3:1), ethanol precipitated and dissolved in diethyl pyrocarbonate-treated water. mRNA differential display was performed with the RNA map™ kit (Gene Hunter Corp., Nashville, TN, USA), AmliTaq DNA polymerase (Perkin-Elmer, Branchburg, New Jersey, USA) and [33P] dATP (0,2µl/20µl PCR reaction of 111 000 GBq/mmol; Isotopchim, Ganagobie-Peyruis, France). 3.5 µl of each PCR reaction was mixed with 2µl of loading dve and denatured at 95°C for 5 minutes prior to loading onto 6% DNA sequencing gel. Gels were electrophoresed at 90 Watts constant power until the xylene dye reached the bottom and dried at 80°C for about 1 hour. All the 20 decamers were used in combination with the four T₁₂MN primers provided with the kit. Bands with differential expression pattern and larger than 200 bp were purified from the polyacrylamide gels and reamplified according to the instructions provided in the manual of the RNAmap™ kit. Reamplified cDNA was ethanol precipitated and cloned into pGEM®-T vector (Promega Corp., Madison, WI, USA). Each clone was assigned a number corresponding to the primer used, position on the gel and number of cDNA fragment within the isolated band (e.g. t18-2-5 was amplified with primers T₁₂MT and AP18, isolated as a second from the top of the gel, and after the cloning fifth colony was sequenced).

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DNA sequence analysis

3 to 6 cDNAs originating from a single band were sequenced by primer walking using ABI Prism® BigDye™ terminator cycle sequencing kit (PE Applied Biosystems, Foster City, CA, USA). DNA sequence data were analysed using the Wisconsin Package Version 9.1 (Genetics Computer Group (GCG), Madison, Wisc.). The nucleotide sequences of all cloned cDNAs were compared with sequences deposited in GenBank, EMBL, DDBJ, PDB databases, and translated DNA sequences were compared with protein sequences of GenBank CDS translations, PDB, SwissProt, PIR and PRF databases using BLAST algorithm (Altschul *et al.*, 1997). The scoring matrix used by blastp search was BLOSUM62 (Henikoff and Henikoff, 1992). Gene homologues in database were considered to be significant when the e-value was <10⁻³ and the high-scoring segment pair identity was at least 20% for amino acid sequence and 50% for nucleotide sequence.

Plasmid construction

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pWRKY11: WRKY11 cDNA amplified from cDNA library with primers EVVRA 28 and EVVRA 29 and cloned in pGEM-tTM(Promega) Pstl and Notl site via intermediate cloning in the pZErOTM vector (Invitrogen)

pWRKY-pGSJ780A: *Bg/*II-digested *WRKY11* sequence was cloned into the BamHI site of the pGSJ780 binary vector (Bowler et al., 1991).

pWRKY-VP16-pGSJ780A: VP16 activation domain amplified form pTETVP16 by primers EVVRA 26 and EVVRA30 and cloned as Xho1 fragment in Xho1 site of pWRKY11.

The WRKY-VP16 fusion was then cloned as *Bgl*II fragment into the BamHI site of pGSJ780A.

Arabidopsis transformation

Arabidopsis transformation was carried out by the floral dip method (Clouch and Bent, 1998). Selection of primary transgenics and progeny was based on transgene expression levels as determined by Northern blot analysis.

Stress assessment:

80 plants of a F₂-progeny of the transgenic line WV4 (construct pWRKY-VP16-pGSJ780A) were grown on MS+Kanamycine for 2.5 weeks. 15 kanamycine resistant seedlings were transferred to plates containing ½ MS, 1% sucrose and 2 μM methyl viologen (=paraquat) or to ½ MS, 1% sucrose for the controls.

Wild-type *Arabidopsis* plants were treated in a similar way (except for selection on Kanamycine).

Performance of plants was followed and pictures were taken after ~3 weeks.

Example 1: Sensitivity of tobacco to methyl viologen

As a first step in studying adaptive responses to oxidative stress in tobacco, we wanted to establish an experimental system in which low doses of oxidant would induce adaptation to higher doses of the same compound. WV, a redox-active compound that enhances superoxide radical (O₂*) formation mainly in chloroplasts, was used as an oxidant. In order to determine MV concentrations suited for the

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induction of adaptation and for the subsequent oxidative stress treatment, sensitivity of tobacco to MV was first determined. Leaf discs were floated on solutions with different concentrations of MV and ion leakage was monitored by measuring the solute conductance. If not scavenged, superoxide generated by MV is converted through redox-reactions into other active oxygen species (AOS) such as hydroxyl radicals that interact with biological molecules and cause oxidative damage (Halliwell and Gutteridge, 1989). Peroxidation of membrane lipids results in loss of membrane integrity that is reflected by enhanced cellular ion leakage. Concentrations lower than 0.2uM MV caused very little increase in ion leakage from the leaf discs in comparison with water-treated controls and no visible damage was seen even after 42 hours of incubation (Figure 1). These concentrations thus seemed most suitable for inducing adaptation to MV. When leaf discs were incubated in MV solutions at concentrations ranging from 0.2-2 µM MV, leaf damage measured as solute conductance clearly correlated with the applied dose of MV. This correlation was more or less linear within this range, suggesting that these doses of MV are most suited for monitoring differences in MV sensitivity between pre-treated and control samples.

Example 2: MV pre-treatment induces tolerance and activates expression of antioxidant genes.

To test, whether exposure to sub-lethal amounts of MV enhances tolerance to higher, normally toxic amounts of this compound, tobacco leaf discs were floated on solutions with less than 0.2 μ M MV and subsequently transferred to solutions within the molar range of 0.2-2 μ M. Increase in tolerance was assessed by measuring the solute conductance. Leaf discs pre-treated with water were taken as non-adapted controls. Protection against MV was most pronounced (40% in the mean compared to water pre-treated control samples) when leaf discs were pre-treated with 0.1 μ M MV for 17 hours (including 8 hours dark period; referred as "pre-treatment") and subsequently treated with 1 μ M MV for 11 hours (referred as "treatment")(Figure 2). These parameters for the pre-treatment and the treatment were then used in all further experiments, unless otherwise stated. The specific conditions required for inducing adaptation were not investigated; yet, it was noticed that both the MV concentration and duration of the pre-treatment were factors that affected the level of protection.

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mRNA levels of several antioxidant genes were tested by Northern analysis during the pre-treatment and the treatment. Both water and MV caused a rapid induction (1hr) of a glutathione peroxidase gene (Gpx) and a gene encoding cytosolic CuZnSOD (SodCc) (data not shown). Gpx and SodCc were only transiently induced in water pretreated samples, suggesting that this induction was caused by the tissue wounding during leaf discs preparation. In contrast, pre-treatment with 0.1 µM MV gave a persistent increase in Gpx and SodCc mRNA. After transfer to 1 µM MV, Gpx and SodCc were again induced in both water and MV pre-treated samples. However, the amount of both messengers remained consistently higher in MV pre-treated samples (Figure 3). The above data indicate that induced tolerance is not just a physiological response but that it involves changes in nuclear gene expression and that GPx and cytosolic CuZnSOD are playing a role in the observed enhanced tolerance of pretreated samples. Cat1 and SodB genes were also induced following the pre-treatment, but their transcript levels declined during the subsequent treatment with 1µM MV and no correlation could be established between their mRNA levels and enhanced tolerance.

Example 3: Expression of a large number of genes implicated in distinct cellular processes is modulated by MV pre-treatment.

In order to identify which genes other than those encoding antioxidant enzymes would show altered mRNA levels during oxidative stress adaptation, reference samples placed in water for 17 hours, or samples, pre-treated with 0.1 µM MV for 17 hours (adapted leaf discs) were compared by differential mRNA display. To increase the fidelity of the differential display results, mRNA from two independent experiments was used to prepare cDNA, and reverse transcription was performed in duplicates for each RNA sample. Amplified cDNA from two separate experiments and two independent reverse transcription reactions were displayed next to each other on the sequencing gel. Eighty primer combinations yielded 243 bands larger than 150 bp that consistently showed differential expression between adapted and non-adapted samples. 202 of them were up-regulated and 41 were down-regulated. Reamplified cDNA fragments larger than 200bp were cloned and 3 to 6 cDNAs from 60% of all bands sequenced. Sequencing data revealed that 50% of sequenced bands contained two or more cDNA species and 30% of bands were redundant. Taking in account this redundancy and

assuming that only one cDNA species per band contributed to the differential expression pattern, the total number of genes with altered expression after MV pretreatment is estimated to be 170. Expression of 16 genes was further analysed by Northern analysis with RNA from an independent experiment. The induction of 12 genes was confirmed, while 4 genes remained uninduced. 3 out of these 4 genes were isolated from bands consisting of mixed cDNAs, indicating that they were not responsible for the differential expression pattern. The fact that expression of most of the isolated genes was reconfirmed by Northern analysis is a good indication of procedure fidelity and suggests that the number of genes transcriptionally responding to MV is close to the number estimated by sequencing data.

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The nucleotide sequences and translations of 167 cDNAs isolated from differentially expressed bands were compared with non-redundant databases. Only 12 cDNAs were identical or highly similar (>90% over the whole sequence) to previously isolated tobacco genes. Of the other 145 cDNAs, 36 were significantly similar to genes/proteins with known or predicted function, and 16 to genes with no assigned function. The high percentage of cDNAs (62%) for which no similarity was found in the database can in part be attributed to the fact that the isolated cDNAs mostly contain 3'untranslated region where sequence divergence is very high. The homologues of isolated cDNAs. of which the expression was tested and reconfirmed by Northern analysis, are listed in Table 2. Data shows that in addition to antioxidant genes, genes encoding chaperones (DNAJ), transporter proteins (MDR), dioxygenases (DIOX), enzymes of carbohydrate (ATPC-L), lipid (Lox2, MFP) and terpenoid metabolism (EAS, VS), regulatory proteins (WRKY11, TPK) and pathogen related proteins (PRB1b, CBP20) are activated during MV induced adaptation to oxidative stress in tobacco. The large number as well as the functional diversity of genes transcriptionally responding to MV pre-treatment indicates that AOS activate a wide range of responses within the plant cells.

Example 4: MV induced genes are regulated differently during the treatment.

Of the antioxidant genes tested, only expression of *Gpx* and *SodCc* correlated with enhanced tolerance of pre-treated samples (Figure 3). To further investigate the transcriptional response of genes induced during adaptation to MV, Northern hybridisations were performed for a subset of identified genes (Table 2) during the pre-treatment and the treatment (Figure 4). The earliest gene induction could be observed already after one hour of the pre-treatment for *MFP* and *Lox2* and is likely related to

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the wounding of the tissue during the leaf discs preparation. Lipoxygenase (Lox) and multifunctional protein (MFP) are both implicated in a pathway leading to lipid breakdown products such as jasmonic acid, and wounding may induce their expression (Mueller, 1997). This induction was transient and was seen in both water reference samples and MV pre-treated samples.

During the first four hours of the pre-treatment there was no discernible induction of gene expression by MV, while during the treatment, the induction was visible already after three hours. The concentration of MV during the treatment was ten times higher suggesting that the timing of induction is concentration dependent. All genes, except *DIOX*, were induced after 12 hours of the pre-treatment with 0.1μM MV, but more detailed time course analysis would be required to determine exact timing of induction. The low level of induction at this time point reflects probably the preceded dark period of 8 hours with no photosynthetic activity. Primary site of action of MV in photosynthesising plants are the chloroplasts (Halliwell and Gutteridge 1989) and active photosynthesis is required for maximal generation of superoxide by this redoxcycling compound. This is in agreement with the further and much stronger induction of the mRNA level on the light during the last five hours of the pre-treatment.

Expression of all genes, except DIOX, was further induced during the treatment with 1 μ M MV and the induction started within the first three hours of the treatment. In the course of the treatment two different expression patterns were essentially recognised. For one group of genes (PRB-1b, CBP20, VS, MDR, DNAJ and WRKY11), expression was induced by a 1 μ M MV treatment in both, the 0,1 μ M MV pre-treated samples and water reference samples as such that the level of transcript remained higher in the 0,1 μ M MV pre-treated samples for at least six hours, which is the time when the difference in tolerance between pre-treated and non pre-treated samples began to be manifested. The increase in transcript levels with time was rather slow reaching the maximum between 6-9 hours in water reference samples, while it was generally 3 hours earlier in MV pre-treated samples. Towards the end of the treatment, the transcript level declined. A similar expression pattern was observed for antioxidant genes GPx and SodCc (Figure 3).

The second group of genes (*EAS*, *TPK*, *Lox2* and *MFP*) was also transcriptionally induced by a 1 μ M MV treatment (except *Lox2* in MV pre-treated samples) but with different kinetics. The induction was much stronger in the water reference samples, so the differences in mRNA level between MV pre-treaded and the water reference

samples diminished. The response was also faster, with transcript levels reaching a maximum within 3 hours (6 hours for *MFP*) in both, water reference and MV pretreated samples. The kinetics of *ATPC-L* expression had rather intermediate character with respect to the expression patterns of the two described gene groups. Together these data indicate the presence of at least two different mechanisms for activation of defence genes by MV.

Example 5: overexpression of WRK11 provokes oxidative stress tolerance.

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Full-length cDNA sequence was obtained by 5'RACE using total leaf RNA and a genespecific 3' primer.

The corresponding gene was designated *WRKY11* because 10 non-identical tobacco WRKY genes were already present in the database.

WRKY proteins are divided into 3 classes: based on type and number of WRKY domains. WRKY family members show only little homology among each other outside of the WRKY domains (Eulgem, Rushton et al. 2000). Database search (blastx on nrprot) revealed only 1 protein that is significantly similar to WRKY11 within the N-terminal part of the protein: StWRKY1 from potato (Dellagi, Heilbronn et al. 2000).

Segregating populations (F2) of *A. thaliana* plants (C 24) transformed with *WRKY11* under control of the 35S promoter (35S-WRKY11) or with *WRKY 11* fused to the *VP16* activation domain under control of the 35S promoter (35S-WRKY11-VP16) were grown on MS media with kanamycine. ~ 3 weeks old seedlings resistant to kanamycine from 3:1 segregating lines (WV4 and WV9 *WRKY11-VP16* transformed lines) were transferred to the solid media containing ½ MS salts, 1% sucrose and 2 µM methyl viologen (MV) or on plates without MV. As control plants untransformed *A. thaliana* plants were used (C24). After 3-4 weeks, phenotypic differences were assessed.

On control plates without MV, no difference in growth between *WRKY-VP16* transformants and controls were observed (Fig 5 A). On plates containing MV, growth of all plants was retarded, however differences in growth and MV tolerance between lines overexpressing *WRKY11* and control plants were observed.

Line WV4 was more tolerant to MV than untransformed Arabidopsis control (C24). However, line WV9 did not differ significantly from control in its growth and MV tolerance (Fig 5, B).

Table 1: list of stress related genes with identification on the base of homology

Clone number	DD+/-	N+/-/=	h mology E<10-3 with at least 20%amin acids or 50% nucleic acids id ntical
			non-redundant DNA and protein s quenc databases (blastx/blastn)
a1-1-14.seq	+		
a1-1-7.seq a10-2-12.seq	+		hypothetical protein [Arabidopsis thaliana] (gb AAD08932)
a10-2-12.seq	+		metallothionein-like protein type 2 Nicotiana plumbaginifolia (gb U35225)
a10-4-12.seq	+		
a10-4-15.seq	+		
a14-1-1.seq	+	=	serine carboxypeptidase-like protein Oryza sativa (dbj BAA04511)
a14-1-3.seq	+		
a14-1-4.seq	+		EDERD 4 Matricario chamomillo (dhiIRAA97069)
a18-1-5.seq a18-1-8.seq	+		EREBP-1 Matricaria chamomilla (dbj BAA87068)
a18-3-2.seq	+		
a18-3-3.seq	+		EIF-5A (eukaryotic initiation factor 5A2) Solanum tuberosum (sp]P56333)
a18-4-6.seq	+		
a19-3-1.seq	+		
a19-3-3.seq	+		
a19-3-4.seq	+		
a19-3-9.seq	+		
a20-1-3.seq a3-2-2.seq	-		ribosomal protein L12 (60S) Prunus armeniaca (sp O50003)
a8-1-1.seq	-		Thousantal protein E12 (003) Fitulus annemada (Sp[000000)
a8-1-2.seq	-		geranyl-geranyl reductase chlP-gene Nicotiana tabacum (emb CAA07683)
a8-1-4.seg	-		early wound inducive gene Nicotiana tabacum (dbj BAA95791)
a9-1-2.seq	+		epoxide hydrolase Nicotiana tabacum (gblAAB02006)
a9-3-4.seq	+		immediate-early salicylate-induced glucosyltransferase (IS10a) Nicotiana tabacum
			(gb U32643)
a9-4-1.seq	+		
a9-5-9.seq	+		
a9-6-11.seq a9-7-1.seq	+		
a9-7-10.seq	+		lipoxygenase LOX1 Nicotiana tabacum (emb[X84040)
a9-7-11.seq	+	 	iipoxygoniaeo Eext Titoettana tababoni (ethib) te te te)
c1-1-3.seq	+		
c1-1-5.seq	+		
c1-2-2.seq	+		
c1-3-12.seq			
c10-3-1.seq	<u> </u>		
c10-3-5.seq c11-2-1.seq	+		
c11-3-1.seq	+	<u> </u>	
c11-3-3.seq	+		caffeoyl-CoA O-methyltransferase Nicotiana tabacum (emb Z56282)
c13-1-6.seq	+		
c13-2-1.seq	+		L19 ribosomal protein Nicotiana tabacum (emb Z31720)
c13-3-13.seq	+		23S 4.5S rRNA genes chlP-genes Nicotiana tabacum (gb J01446)
c13-3-6.seq	+		the state of the s
c14-1-60.seq	+	ļ	glycolate oxidase Lycopersicon esculentum (pir T07032)
c14-2-10.seq	+	ļ	ribosomal protein L35-like (60S) Arabidopsis thaliana (emb[CAB85998)
c14-2-15.seq c14-3-4.seq	+	 	ribosomal protein L33-like (60S) Arabidopsis thaliana (emb[CAB63996) ribosomal protein L23a-like (60S) Arabidopsis thaliana (emb[CAB75762)
c14-5-1.seq	-	 	predicted protein Oryza sativa (dbj BAA83350)
c14-6-11.seq	+		predicted protein Arabidopsis thaliana (pir T02387)
c14-7-4.seq	+		
c15-1-2.seq	+		
c15-1-4.seq	+	+	pathogen- and wound-inducible antifungal protein CBP20 precursor Nicotiana
-45 44 6	ļ ,	 	tabacum (gb AAB29959)
c15-11-2.seq	+	<u> </u>	
c15-11-4.seq c15-2-8.seq	+		hypothetical protein Arabidopsis thaliana (emb CAB88533)
c15-2-6.seq	+	 	hypothetical protein Arabidopsis thaliana (gbIAAF63779)
c15-6-2.seq	+		431,000
c15-6-3.seq	+		
c15-7-1.seq	-		
c15-8-5.seq	-		
c17-3-1.seq	+		
c17-3-5.seq	+	<u>L</u>	

W O 03/012090			
c17-5-5.seq	+		
c17-5-8.seq			
	+		
c17-6-2.seq			DNA London III. Applidancia Abeliana (ambiCARSCO70)
c18-1-2.seq	+	+	DNAJ protein-lik Arabidopsis thaliana (emb CAB86070)
c18-2-1.seq	+		CCT (chaperonin containing TCP-1) b subunit Oxytricha nova (gb AF188130)
c19-2-11.seq	+		
c19-3-10.seq	+		
c19-4-19.seg	+		
c19-4-22.seq	+	- 1	
c19-5-1.seq	-		
			
c19-5-4.seq	-		
c19-6-3.seq	+	<u> </u>	
c19-7-4.seq	+		putative translation initiation factor 2B beta subunit (NIFb) EIF2B beta homolog
1		1	Nicotiana tabacum (gblAF137288)
22 4 40 222	-		
c2-1-10.seq			
c2-11-14.seq	+	i	
c2-11-2.seq	+		
c2-2-1.seq	+		
c2-2-3.seq	+		
c2-4-1.seq	+		
c2-5-6.seq	+		
	-		
c2-6-5.seq			non-sucrose-inducible patatin precursor -strand Solanum brevidens (gb U09331)
c2-7-1.seq	+		non-sucrose-muucipie patatin piecursor -strand Solanum previdens (goloosoot)
c2-9-14.seq			
c20-1-4.seq	+		DNA- binding protein (pabf) Nicotiana tabacum (gb U06712)
c3-2-4.seq	+		· · · · · · · · · · · · · · · · · · ·
c3-3-6.seq	+		
c3-4-1.seq	-	1	
c4-1-2.seq	+		
c4-3-3.seq	+		
c5-1-2.seq	+		
c6-8-13.seq	+		
c6-8-4.seq	+		
c6-8-9.seq	+		
c7-1-2.seq	- 1		
c7-1-6.seq	-		
c7-3-10.seq			
			hypothetical protein Arabidopsis thaliana (emb CAB62623)
c7-3-3.seq	-		hypothetical protein Arabidopsis trialiana (embloabozozo)
c7-3-9.seq	-		
c8-1-5.seq	+		
c9-1-4.seq	+		hypothetical protein Arabidopsis thaliana (dbj BAB08809)
			putative ABA-repsonsive protein Arabidopsis thaliana (dbj BAB11190)
g10-1-1.seq	+		putative AbA-repsonsive protein Alabidopsis triadiana (db/bA-repsonsive protein Alabidopsi triadiana (db/bA-repsonsive protein Alabidopsi triadiana (db/bA-repsonsive protein Alabidopsi triadiana (db/bA-repsonsive protein A
g12-1-21.seq	1 - {		hypothetical protein Arabidopsis thaliana (pir T01731)
g12-1-5.seq	-		Putative membrane related protein Arabidopsis thaliana (gb AAD38248)
g14-2-4.seq	+	+	vetispiradiene synthase Solanum tuberosum (gb AAD02223)
			venopinations symmetric section (3-)
g14-3-10.seq	+		/- UTAAA/T)
g14-3-22.seq	+		hypothetical protein Spinacia oleracea (pir T09217)
g14-3-3.seq	+		Sequence 162 from Patent EP0953640 Nicotiana tabacum (emb AX014606)
g14-3-4.seq	+		HR associated Ca2+-binding protein Phaseolus vulgaris (gb[AAD47213)
			the good and a sure of the sur
g14-3-7.seq	+		
g15-1-37.seq	+		putative golgi transport complex protein Arabidopsis thaliana (gb AAF16568)
g15-2-2.seq	+	=	ubiquitin Nicotiana tabacum (gb U66264) able to induce HR-like lesions
g15-3-11.seq	-		Sequence 7 from Patent EP0953640 Nicotiana tabacum (emb AX014451)
		-	
g15-3-7.seq			
g15-4-1.seq	+		
g17-2-13.seq	+	+	WRKY DNA binding protein Solanum tuberosum (emb CAB97004)
	+		
g17-3-2.seq			putative ribosomal protein L18 (60S) Arabidops thaliana (gb AAF26138)
g18-4-7.seq	+		putative ribosomai protein LTO (000) Arabidops thanana (gujeen 20136)
g18-5-1.seq	-	L	
g18-5-12.seq	-	·	
g18-6-12.seq	+		
			
g18-6-5.seq	+	L	
g18-7-5.seq	+		
g18-8-7.seq	+		
	-		unknown protein Arabidopsis thaliana (gb AAF23197)
g19-1-5.seq			Children broton, and and about an annual (201 - 2 - 2 - 1)
g19-1-6.seq	+	L	
g19-1-7.seq	+	1	putative protein Arabidopsis thaliana (emb CAB82697)
g19-2-1.seq	+		
		 	
g19-2-9.seq	+		
g2-1-2.seq	+	+	5-epi-aristolochene synthase Nicotiana tabacum(emb Y08847)
g20-2-20.seq	+	1	hypothetical protein Arabidopsis thaliana (gb AAF14679)
, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			

g20-2-29.seg	+	T	
g20-2-31.seg	+		
g3-1-1.seq	+	 	ankyrin-like protein Arabidopsis thaliana (dbj BAB10271)
g3-1-4.s q	+	+=	ADP-ribosylation factor Capsicum annuum (gb AAF65512)
g6-2-13.seq	+	+	leucoanthocyanidin dioxyg nase 2, putativ ; 51024-52213 Arabidopsis thaliana (gblAAG21532)
g6-3-7.s q	+	+	ATP citrate lyase Arabidopsis thaliana (dbj BAB09916)
g6-4-4.seq	+		
g6-4-5.seq	+		ATP-dependent protease proteolytic subunit ClpP-like protein Arabidopsis thaliana (dbi BAB09831)
g7-1-1.seq	+		RNA-binding protein MEI2 (meiotic regulator), putative; 36123-32976 Arabidopsis thaliana (gb AAG12640)
g7-1-4.seq	+		
g9-2-2.seq	+	+	P-glycoprotein-like protein Arabidopsis thaliana (emb[CAB71875)
g9-2-6.seq	+		
g9-3-17.seq	+		
g9-3-4.seq	+		
g9-5-5.seq	+		
g9-6-1.seq	+	+	lipoxygenase Solanum tuberosum (gb AAD09202)
t12-1-7.seq	+	+	serine/threonine/tyrosine-specific protein kinase APK1A Arabidopsis thaliana (splQ06548)
t12-2-1.seq	+		chitinase class 4 Vigna unguiculata (pir S57476)
t12-2-18.seg	+		
t18-2-5.seq	+	+	basic PRB-1b Nicotiana tabacum (emb X66942)
t18-3-2.seq	+		
t18-3-6.seg	+		RNA- or ssDNA-binding protein Vicia faba (pir/T12196)
t18-4-18.seq	-		ADP-glucose pyrophosphorylase small subunit Solanum tuberosum (emb X55650)
t-2-1-1.seq	+		ubiquitin carrier protein Lycopersicon esculentum (sp P35135)
t2-1-3.seq	+		Hypothetical protein chlP Nicotiana tabacum (splP12204)
t2-6-3.seq	+	1	
t7-1-12.seq	+	=	Hypothetical protein Arabidopsis thaliana (gb AAF26468)
t7-1-14.seq	+		t7-2-4.seq + intron
t7-2-4.seq	+	+	Multifunctional protein of glyoxysomal fatty acid beta-oxidation Brassica napus (emb[AJ000886)
t7-4-7.seq	+		putative glutathione S-transferase; 80986-80207 Arabidopsis thaliana (gb AAF15930)
t7-4-8.seq	+		
t7-5-4.seq	+		
t7-5-5.seq	+		
t7-6-4.seq	+		

DD+ = induced on differential display gel
DD- = repressed on differential display gel
N+ = induced on Northern
N- = repressed on Northern
N= = constant on Northern

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Table 2. Genes isolated by differential display with induction confirmed by Northern analysis.

Columns refer, respectively to the clone number; the name of the predicted gene, the length of isolated cDNA including both primers; the length of deduced partial protein sequence; the (putative) homologue with highest e-value identified in the database; accession number of a (putative) homologue; percentage of the amino acid sequence identity (superscript indicate homology of the same segment to similar domains localised upstream ⁽¹⁾ and downstream ⁽²⁾ in the homologous protein); the length of the high-scoring segment pair(s) identified by blastx homology search.

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HSPS length (aa)	47	84	2	65	48	88	95	85	17	46	82	98
%sequence identity (aa)	100%	%86	100%	100%	%26	75%	68% ⁽¹⁾ 91% ⁽²⁾	%08	100%	61%	36%	94%
Accession Number	emb X66942	gb AAB29959	emb Y08847	gb AAD02223	db] BAB09916	emb[CAB86070	emb CAB71875 6	gb AAG21532	gb AAD09202	emb[AJ000886	sp Q06548	emb[CAB97004 5
(Putative) homologue	pathogenesis-related protein 1b, PRB-1b (Nicotiana tabacum)	pathogen- and wound-inducible antifungal protein CBP20 (clone cbp20-52) (<i>Nicotiana tabacum</i>)	5-epi-aristolochene synthase (clone str319) (Nicotiana tabacum)	vetispiradiene synthase (Solanum tuberosum)	ATP citrate-lyase (Arabidopsis thaliana)	DnaJ-like protein (Arabidopsis thaliana)	P-glycoprotein-like protein (Arabidopsis thaliana), nucleotide binding fold NBF2	Leucoanthocyanidin dioxygenase 2-like protein (Arabidopsis thaliana)	Lipoxygenase (Solanum tuberosum)	Multifunctional protein of glyoxysomal fatty acid beta-oxidation (Brassica napus)	Protein tyrosine-serine-threonine kinase APK1A (Arabidopsis thaliana)	WRKY DNA binding protein (Solanum tuberosum)
Peptide length (aa)	48	84	8	99	49	88	96	96	19	55	75	87
cDNA length (bp)	448	508	228	382	397	397	505	525	569	413	361	548
cDNA/ gene name	PRB-1b	CBP20	EAS	S/	ATPC-L	DNAJ	MDR	DIOX	Lox2	MFP	TPK	WRKY11
Clone number	T18-2-5	C15-1-4	G2-1-2	G14-2-4	G6-3-7	C18-1-2	7-2-69 20	G6-2-13	G9 -6-1	17-2-4	T12-1-7	G17-2-13

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 - Willekens, H., Villarroel, R., Van Montagu, M., Inzé, D. and Van Camp, W. (1994) Molecular identification of catalases from *Nicotiana plumbaginifolia* (L.) *FEBS Lett.* **352**, 79-83

CLAIMS

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1. A method to isolate stress regulated genes or gene fragments comprising

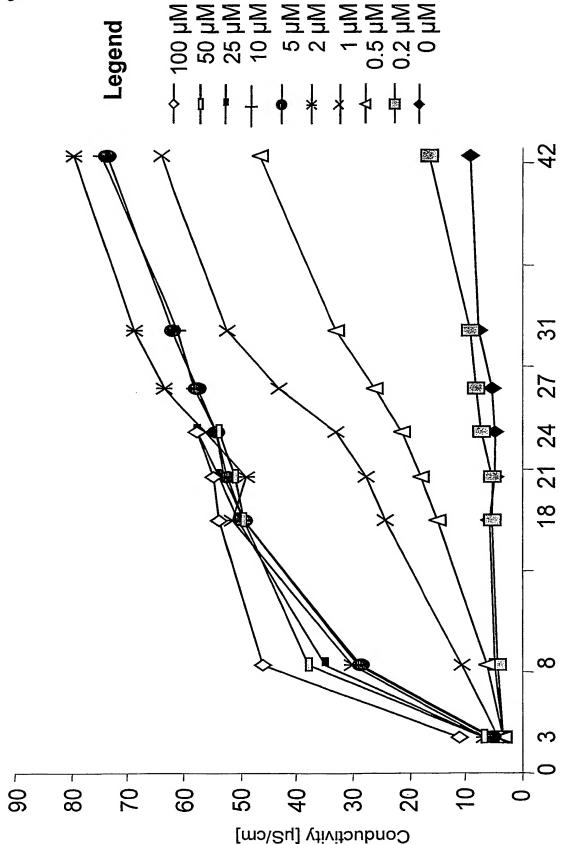
- (a) isolating plant material
- (b) inducing stress adaptation in said plant material
- 5 (c) checking differential expression between stress adapted and non adapted plant material
 - (d) isolating differentially expressed genes or gene fragments.
 - 2. A method according to claim 1, where by said induction of stress adaptation is obtained by a methyl viologen pre-treatment and/or treatment.
- 3. A method according to claim 1 or 2, whereby said plant material is tobacco leaf material.
 - 4. A method according to any of the claims 1 3, whereby said isolation of differentially expressed genes or gene fragments is carried out by PCR reaction.
 - A gene or gene fragment, obtained by a method according to any of the claims 1 –
 4.
 - 6. A gene or gene fragment, according to claim 5, comprising a sequence selected from any of the sequences from SEQ ID N°1 to SEQ ID N°167.
 - 7. A gene, according to claim 5, encoding a protein comprising SEQ ID N° 169.
 - 8. A gene according to claim 7, comprising SEQ ID N° 168.
- 9. The use of a gene according to claim 5, or a gene that is at least 60% identical, preferably 80% identical, more preferably 90% identical to said gene, to modulate plant stress tolerance
 - 10. The use of a gene comprising a sequence selected from any of the sequences from SEQ ID N°1 to SEQ ID N° 167, or a gene that is at least 60% identical, preferably 80% identical, more preferably 90% identical to said gene, to modulate plant stress tolerance.
 - 11. The use of a gene encoding a protein comprising SEQ ID N° 169 to modulate plant stress tolerance.
 - 12. The use of a gene according to claim 11, whereby said gene comprises SEQ ID N° 168.
 - 13. The use of a gene fragment according to claim 5, whereby said gene fragment is a promoter, to modulate plant stress tolerance.

14. The use of a promoter derived from a gene according to claim 5 or 6, or from a gene that is at least 60% identical, preferably 80% identical, more preferably 90% identical to said gene, to modulate plant stress tolerance

- 15. The use according to claim 9 or 14, whereby said stress is oxidative stress.
- 5 16. The use according to any of the claims 9 15, whereby said plant is tobacco.
 - 17. A vector comprising a gene or a gene fragment according to any of the claims 5 8.
 - 18. A method to modulate stress tolerance of a plant cell or plant, comprising the introduction of a vector according to claim 17 in said plant cell or plant.
- 10 19. A plant cell or plant, comprising a vector according to claim 17.

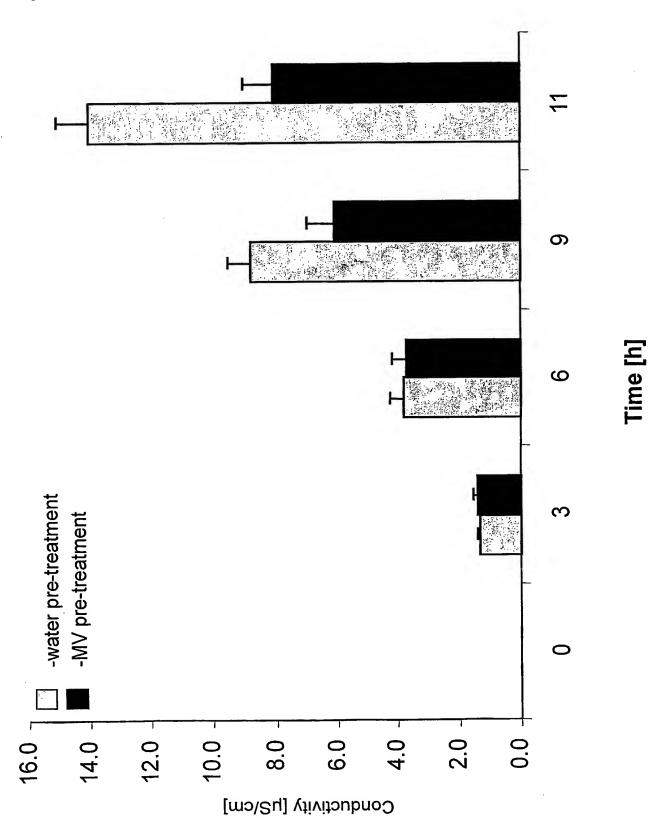
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Fig. 1



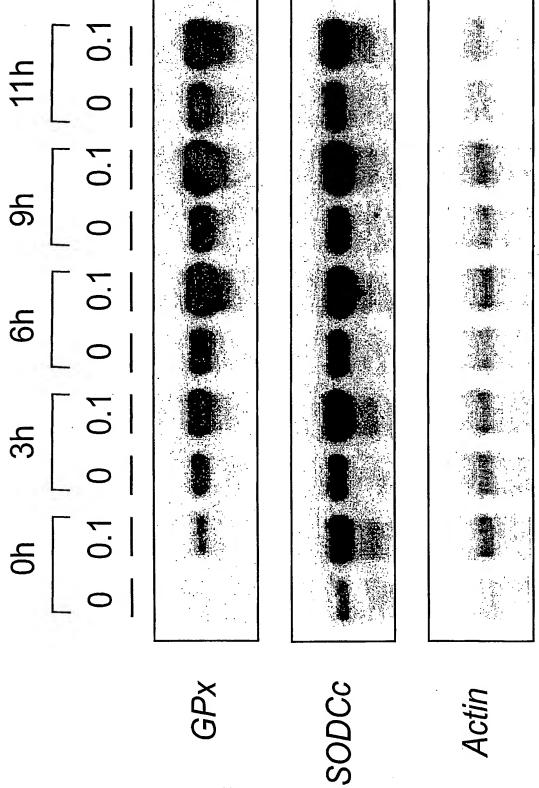
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Fig. 2



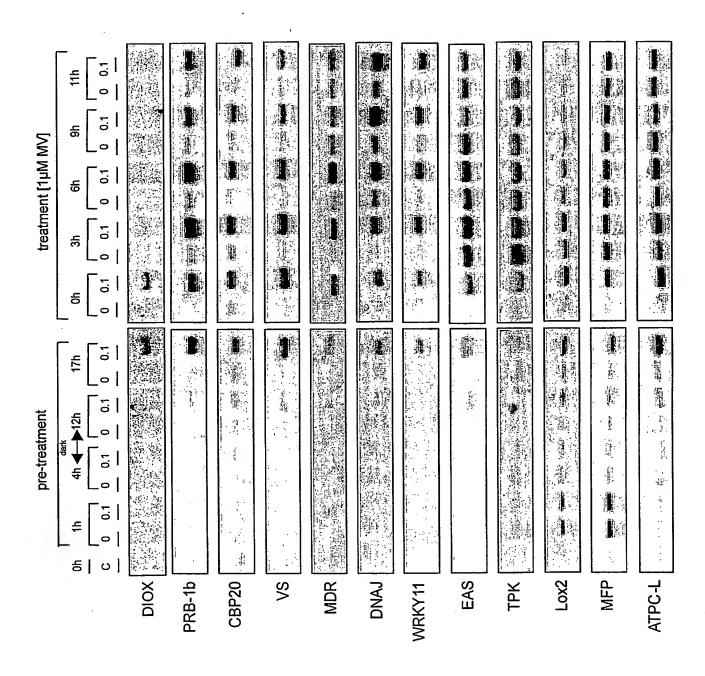
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Fig. 3



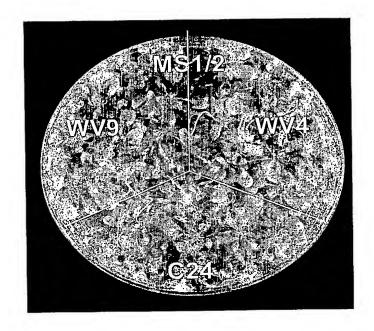
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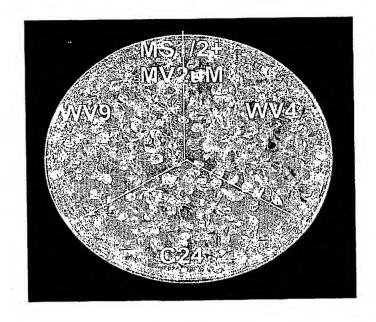
Fig. 4



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Fig. 5





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      O-methyltransferase 3' [I]
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aaaagettat aagaacaaga agtaettgee tettgaeete egteecaaga agaetaggge 240
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<223> plasmid c15-11-4; Arabidopsis genomic homology

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<223> plasmid c17-5-8; Arabidopsis genomic homology
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atttgcagcc ttggacacac ctcagcaaca gaatggaacn tcaacaacac taanaanttg 180
cacacctaaa tccaaaacaa aaagactcga ctccgtatca naaantangg tttacntgaa 240
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<223> plasmid cl9-4-19 ; Arabidopsis genomic homology
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agaaacagca ctctccactt gtatctcagg aatgcactat aagaaaatct antatactan 240
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aaaaattaca acacttggga actgaanaac cttanctgac cccagaaaac cattaaaggt 180

aatatagcgc atctttacac ggttgtgaan atcacaaaat atcctcaatt tgttgcctaa 240 242 ct <210> 75 <211> 257 <212> DNA <213> Nicotiana tabacum <220> <223> plasmid c19-7-4; homology with putative translation initiation factor 2B beta sub. NIFb <400> 75 ataaactata ntaccattta gttgttgata atacgaatga ataaaccatt cgacaactta 60 acttttcagt caacaatagc atacgtgttg tctaataata ccacaaagga aaaccaccat 120 caagtagtac totgoatato ogaaatoaca aaactocago acaaatotaa totoanaato 180 aatctacaaa ctccaaaaat cgcgatgctc tcttcatctg tttattgcag tcagtataat 240 257 gtaggtgcaa catcttg <210> 76 <211> 384 <212> DNA <213> Nicotiana tabacum <220> <223> plasmid c2-1-10 <400> 76 qtqcaqtaaa ctqaataggt tgacagagct agctgccaga tgactcttca tgcggtaggg 60 tttttcttat attactgcca tacagtattg gagctggaga tatcaagacc gtgctagctc 120 tgctqattaq ttgtccgtat agatgacagt gatacataag ctgacttgga atccaagtat 180 ctggtctacc acaattgatt ttctttggga tttactcaca atattcttaa acgatttttg 240 ccggataaat gcaatattca ttgattgtaa tcaatcacta caaggaggat gaagaatata 300 ttcttaaatg atttttgcca gataaatgta atattcatct atatggatag atgaattctt 360 384 gatcaaatgt aagttcatgt cgat <210> 77 <211> 181 <212> DNA <213> Nicotiana tabacum <220> <223> plasmid c2-11-14 <400> 77

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ataacacata tettttgtge ttgattttaa aatacatgag gtgtatttge egttgagtea 120
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aagttgttgg aatcgtatta attttgttag ttaaaggcgg atcaatcaat atatctttcc 180
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ccttataggt ttttgttcat tatctctggt attccttgtc aaagtacatt atgatggcag 180
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<211> 356
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tttgttctta ttagtaataa gtttgttaag ttatcctttc acaaatgata tacagtattg 240
gtgtgaggtg tgtgagggtc atattcttgt gtattaattg ttgcaatgca acgtgtaatt 300
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qctqqtqqtt qttaacttqt cctagattca ctctcactct cattgqtqtg gtccctqtqc 180
tagtgacggg tcttattgtg gctctttaga gttgatgtta tatttactct acctatctgt 240
tgaagtttat ccaattggta tactttttt gggttgttt aacaaagtgc tattcgaatt 300
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tgtaatttca atttcgatca aaccacctta aatctgct
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<223> plasmid c2-5-6
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aggeactica ggtcctcctt gcacgggttg agagcttcca acagatttcg gagattcact 180
aggtagetge ttggcatteg cageceaatg ettetecete tatettattt teteetattt 240
tagttctgta atagactatg tagactcttt ctgttttaaa tcggttagta gatattcatg 300
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gtggagaagt aaataggatt ttatatctaa tattattgcc tttcatagtt ctcagagtat 180
atgtgtagaa caagcacagc tgcaaattgt tattactaat tttatggtgg aaatctgttg 240
aaagttattt tctttt
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<211> 254
<212> DNA
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<223> plasmid c2-7-1; homology with patatin 3'-strand
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ttttggctta gaaggatgat tttatttatt taacacaacc aaaagtctac ataatcctta 180
gcatatttca aatttacata gagggatatt tctattgaaa tttatccctt aacgttacaa 240
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gcgcttattc ttta
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<213> Nicotiana tabacum
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<223> plasmid c2-9-14
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gcattttgct tctgttaaca tggacattat ggatttgtaa attcaactga ctacttgtac 120
acgtctctct ggacattcgg gttattactt ggtacaagtt aataacactt atgctctctc 180
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ttattttatg ctttctgatg aatattcctt ttccctctg
<210> 86
<211> 337
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<223> plasmid c20-1-4; homology with DNA-binding
      protein (pabf) [I]
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aagggggaga caaaatcatg ctcgtgggct atatgtactg ttgtttgagt atgttgaatg 240
gatggaaatg cctttgttag atagatgtat aatgccggca ttatccctca tcaacagttg 300
cctttgcaaa tgtcgtaaaa gcatttgaat tttattg
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ctttctaatt taaagaagga tagaagttca gtgcactctg ctcacaagat gtagtacaag 180
gattettgaa eeaaggattt tgatggaett eatgttgaga ttggaaaact gaatteatta 240
ctggagatca ttgttcatgg ccctataaat ttgaaatttc aaagatacaa atcaaattac 300
                                                                   337
ttatatgtgg catacaacaa gacactacta atacata
<210> 88
<211> 92
<212> DNA
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<223> plasmid c3-3-6
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ttgtcaagta ccgaaaaaac gtgggttggc tacaaaagtc ttaacctggc tagctagcta 240
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cctgctactg agtatct
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gctcttgttt gtattgggat tatttaatta tatttgagat ttataattta ttaaaggcta 180
atcqaatagt gttgactgat gtttggaaaa tgtcatcaga tatcaatgtt ggaagccatt 240
tagctcagta aaattatttt aactaaatca aaagaataaa atactatagg ttggagtaaa 300
taagttgtta atggtagtgt ttttctattt agtcatttgg gatta
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<210> .91
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<212> DNA
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tttggatagt tatggtttct aacttatgta ttagatcatt ttaacaagca gcacagagat 180
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caaattgttc act
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<213> Nicotiana tabacum
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<223> plasmid c5-1-2
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attotacaat qtttccaaqt tatatotgot tttaatogtt totgottgta gottaattgt 180
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<212> DNA
<213> Nicotiana tabacum
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<223> plasmid c6-8-13
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aactcaaact aataaataag gaaactgttt atacagcttt tggaaagcta acccaataag 180
atttggtcat aagtagatgg gttatgttca gttttgagca ggcaatctct ctgaatggaa 240
tqttqttcaq cctqccccta ttqaqaqqaa qaqqacttct tatttttctt aaacccataq 300
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cttagaagtg aaagtctcaa ttgtattgac tatgtaatgt cgtatatatc agtgttttaa 240
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<211> 338
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gtttctttac cqtcctttac atctgaaggc aacttagcat aggagttctt aaatgtatca 300
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aacttcacat ggtcgcgtgg tgttgtgctt tgtgataaaa tgtattgtgt atttatcatc 300
                                                               341
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<210> 98
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tgacaagtac tttcataccc tgctcaaagc aatatgtgtt ttctcgtact tggaagttaa 180
ttttgctgtg gaacaactct tgttagetta gtgttgtggg gtgagetata actcggeetg 240
tgtgatttgt tacatttggt tgagcatttt ctcttatata agaagagaca gtgaggtgtc 300
                                                                   314
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<211> 276
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid c7-3-3; Arabidopsis genomic homology
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qaqaqtaqaq cqtaaaatqq ctqcaqttnc taggqataqa gcatqgqcaq aaaqactgqc 120
agaactgaag aagctcgagg aagagaagaa ggcagccatg gcttgatggt tattgaacag 180
agtttngatc tgttaatttt ctctcttgtt tttgagagtg aaaaatatat taatccctta 240
                                                                   276
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agangtgact cccntntctn tgtcngnata tntntattgg ngggggntnt tttagnattc 300
cagtneatte eganatatag ateneanatt nenatanntn taenanngeg eeceegeneg 360
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gatttctaca aagcttattt ttggtgaatg cttgtgttgt gtgtaatact tcaaccccat 180
ggaaatgcta cgtttattag ctcgtgctgt ggcacccaaa tgaatcttga ttgtgtcatg 240
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<213> Nicotiana tabacum
<220>
<223> plasmid c9-1-4; homology with Drosophila heat
      shock protein 82
<400> 102
gaagtcgagg accgtgccca acggtcagca attacaagag taaatgcaga tgatgttcgg 60
gtcactgtat ccgcacctgc agctcgtgga gaagctaaca atgaacttat ggaattcatg 120
ggtcgagtac tgggtctgaa actatctcag atgactctcc aaagagggtg gaatagcaaa 180
tcaaagcttc ttgtagtgga ggatttgaca gctagacaag tatatgagaa actcttggaa 240
gctgcccaac cttgagatgg ctccctgatc cttttcttct ttgtcatttt ttccatgttt 300
                                                                   346
gtaacattgg atttttagtt tcataaaatt gaattcagtt gtcttt
<210> 103
<211> 360
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g10-1-1; Arabidopsis genomic homology
<400> 103
gaacgagaac aaaccatctc aaaagtacat cgagatagtg actgaagata attttgaatt 60
ttggttcatg ggctttgtac gatatgaaaa agctttcttg aatttacaaa aggctatttc 120
catcacgaat tagctagctg ttaggcatta gaatttttag ggttttaaag aggattcata 180
attetqtaat tgttettttt teettattaa atgttgaact ggtageatet aatetatget 240
```

tgttcatcat tttctttct ctcaacggaa gaggatttga gatttatgag aattgaattt 300

tgtagattct gaaatttaat gaatttctca acatatatat aagatttaga ccaaagttac 360

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<210> 104
<211> 556
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g12-1-21; Arabidopsis genomic
      ABA-regulated gene cluster homology
<400> 104
ggtgggattt gactatgcat atcgcaaagc aatgaattcg actatgaaat tcatcacaag 60
ctcaaaqaac aaqqcqtata catttttaq aacqactacc cccqatcact ttqaqaatqq 120
tgaatggaat acgggaggtt attgtaatag aacaggaccc ttcaaacaag atgaggttga 180
cattggttat gtagatgagg tgatgcgcaa aattgaatta gaagaattcg agagtatatc 240
gagaacagaa totgcagaca ggttgacaat gaaattgtto gataccactt tootttogot 300
gctgagacca gatgggcacc ctggagtcta caggcaatat cagccatttg ctaaagaaaa 360
tatgaacaaa aagattcaga atgattgtct acattggtgc ttgcccggcc caatagattc 420
gtggaacgat gtaatgatgg aaatgttgtt caccagttga aaatggtgtg acattagatt 480
ttgattttgc tcccacaatt gtattgttca tctgcaaaag atggttgcac actatttttc 540
accattgttt cctctc
                                                                  556
<210> 105
<211> 579
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g12-1-5; Arabidopsis membrane related
      protein CP5 homology
<400> 105
tattattcaa gttggtatat tggagaagtg gaatcaagta gaggtaacag ccagccgacg 60
cgatqtqaag tgattctatt ccatcatqaa gatatgggca tcccatggga aattgcaaaa 120
tttggggtaa agcaaggtat gtggggagct gtgaggaaga ttgagcgggg attccgtgcc 180
taccagaaag ctaaagcatc tggcttgaaa atatctcatt gtgcttttat ggctagagtt 240
aatacaaaaa ttgatcgaga atacttgaag tcaatggaag atgatgagga ctcatctgaa 300
actgaattgc aagettcacc tgcaaaacct gagggcatga acataccaaa gctgattatc 360
attqqtqqag ctgtggcagt tgcttgtacc cttaatcaag gaatcttacc caaggtgctt 420
ttqtttaatg ctgtgaaaag gtttggaaat ataggaagga gagcatgtcc aaggacatga 480
catttgattc atgcgtgcat tgcgcatttg ttttttccct gtttaagcat tcacttttaa 540
```

<210> 106

gctctttata tatttaaaac aagcaagtgt tattttgtc

579

```
<211> 358
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g14-2-4; homology with vetaspiradiene
      synthase PVS4 (sesquiterpene cyclase)
<400> 106
gatagcatgg aaggatgtga atgaaggaat tettegaeca aeteetgttt etacagaaat 60
tctcactcgt attctcaatc tcgctcgtat tatagatgtc acttacaagc acaatcaaga 120
tggatacact catccagaaa aggttctaaa acctcacatc atcgctttac tggtggactc 180
cattgaaatc taaaccattg agtgettttt teateteggt gategtttta tttttatttt 240
taaataaagg atcagaactg tgtttctgtg ttcctcttta tataagcaag ttgagtttcc 300
tacttctgtt caaaccctgt gtttgttctt ggcgtctgaa taatataatt ttgtttgc
<210> 107
<211> 264
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g14-3-10
<400> 107
caaagataaa gaaggctgga gttgtaagac aggagcttgc taagcttaag aaggacgctg 60
cttaagaact ctttgattag tgagatttgt atgataggag ttttggaagt cgttgtgttt 120
tgcttttaga ttttggttca ttactggcaa gtcatttggt ttcatctttg gtgtcattga 180
agactcctag aaatcaattt cccaatagtt ttcatttgnn ttatgatggt gaacattctc 240
                                                                  264
ttcgcagaca cttcattttg ttgc
<210> 108
<211> 211
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g14-3-22; homology with orf 03 A.
      thaliana
<400> 108
cttccatcaa gcagggactg gttgggggac tttatggtgt ggaaaccagc agttggtatg 60
gagaatagcc aatcattctg ggcaatttta acaatatgga tagctttggt tggagctgca 120
ctctttttgc aaaagtgaat catatacaag taaagctgtt tattgtctag ctttctattc 180
                                                                  211
tttattqgta tatatagtct gatgtgtatt g
```

```
<210> 109
<211> 262
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g14-3-3; homology with sequence 161 from
      patent EP0953640
<400> 109
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taattgtgga tttatattgg gttgttcatt cagaaagctt tgccaagtaa cttagaatta 120
gtgtttacat tttgatgtct ttgttttgat attactaaga agaaaagata ttggggaaaa 180
aagaaagcca gaccactgaa tggcaggtct gatatgaaaa ctggccatgt atagaaggat 240
                                                                   262
atttcqttta tttcattttt tg
<210> 110
<211> 265
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g14-3-4
<400> 110
gcttcaagtg gatgatgatg atattaaggc catgattaaa ttgggccgtg gtgatgaaaa 60
tggtggtggt gtcacctttg aaggttttct ccaaattttg tctctttgat ttgttgcttt 120
gatgacgatg ataaatgtca gattaggtga acaagttttg gtttactttg tatttttcaa 180
tgatttgttt tactgtgctg cttcatatgc tattggctat tccgagaatt ctatttgaaa 240
acaaagaaga aaaagagttg ttccg
<210> 111
<211> 260
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g14-3-7
<400> 111
atgaagaaga agagggcggt ggtgatgact acattgagtt tgaggatgaa gacattgaca 60
aaatctaaat ctgaacgcaa agctgctgtt actgaggtcc gttataggcc tttctaatgt 120
ttttgtggag ctttttccat aaacattgag agtgtatctt gtgtatcgtt tgaagttatg 180
tatcaaactt tgtgcattgt gagttttgta ttagatttat gcttccatga aatgaatgca 240
                                                                   260
atattctagc tggtgtctac
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<210> 112
<211> 469
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g15-1-37 ; Arabidopsis genomic homology
<400> 112
atattectqq aaacatetca acttgcatca tececaette gteaagatet acegecaagt 60
gtcatactgc accatcttta ctcacgcggg cctgaagaac tacaatcacc attgcaaaga 120
aataqactta ctccqacqca qtattcactc tqqatqqatt cacaagggga ggaccaaatc 180
tggaaaggta ttaaagctac tctggacgac tatgctgcta aggtacggtc aagaggggac 240
aaggaattta gtcctgtcta tcctttgatg ctagaaatcg gctcttcttt atctgggaat 300
cgttagagga gctttgagag aatgcaaagc tcaaatcatc ttctcttggt atatgccctt 360
ccccatattt ttgtttcaat aatattgtca cagatgaaca catagcagac cgttatctat 420
gtttcgttta gtgtcttact ttctttatat attttacctc aattgattg
<210> 113
<211> 350
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g15-2-2; homology with ubiquitin [I] able
      to induce HR-like lesions
<400> 113
gttgatgtcg ttgttgtcgtg ttgattgact gtgtctgttt ctggttgtgg tcgtgatgtg 60
ctttgtctac tgaggtctca aagatgttct atgctatttc tgtttgctgt ttctcttatg 120
ttctctgttg tgaataaaga ttccgaattc tgtcctaaaa aaaaaaaaat gaagtttatg 180
tatattggaa gaagcattgg tgtcgtcacc aagtcccatt tgatatatgg ctgtgttttt 240
gcttggctaa tttgtgttta aactttcttt ctatctgtgc tcaatatact cctgaacaga 300
ctgatgtacg attttaaagc tatgtatgta taaactctct tatcttttgc
                                                                  350
<210> 114
<211> 345
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g15-3-11; homology with sequence 7 form
      patent EP0953640 [I]
```

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<400> 114
gtggatgaag ttaaggtgac ccctgttgct tagaagtaca cagagctttt gtaatggtca 60
atagagtttt ttgcaatgct aatttcatac ttattaagct accactgtga ggcaattgct 120
gtattttacc tatgtgattg ctttaaacta tgaattagat gcctgctgtg agacttgtgt 180
actattgctt ttaaqqaagt gtggatctag ttgaacttcc tctcctttac tatgtgcact 240
ttgatcttga ttcttagata gtcaagaagt aatatataaa attgtactac tatatttcaa 300
atttttcatg tttcttgaag gatgaaatat aaatgagtta gtacc
                                                                  345
<210> 115
<211> 344
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g15-3-7; Arabidopsis genomic homology
<400> 115
gatacatgga atgagttagt gtttgatete atagggagag acttecagag tagacagage 60
aatgetteat aagaagaagg atcettaatg ctaaaaaaca ttttttgtge ttetacagea 120
cagctacggg aagattattt atctctctcg aatggagttt agctttttag ttactttaga 180
tctcttgttg tagctggtgt tgtaatctat gtttagatat ccacggtaag ataattccta 240
agttacacga aattttcaca ggtctcaagt atgtgtgcag ggatatttaa ctaaatacaa 300
acgttttctt tgcaataaaa tatttcatct gatttttccc tcgc
                                                                  344
<210> 116
<211> 301
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid q15-4-1
<400> 116
tgaatgttta atgttagaaa gtgaattact ctctttatgt ggtgtctgaa catatgttca 60
acattactct tcaaattacc aataattaat agtgcgacaa gttataggtt ataggttgat 120
gaaaaattgt ttccatcttg taaattatag tgctaaattt atcacacatc tgtgtgcacc 180
tatattataq tttctgcttt cattqaaaat gaqtttcaaq ttttctaqtq qaattqgata 240
tgtagtatag aagttggagg gttgcttttc attcttttga aagggtaaag caaacttaag 300
                                                                  301
<210> 117
<211> 525
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<212> DNA

<213> Nicotiana tabacum

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<220>
<223> plasmid g17-2-13 ; homology with wrky (zinc finger
     DNA binding protein)
<400> 117
aaqtqqatat tttqqatgat ggttatagat ggaggaaata cggacagaag gctgtcaaga 60
acaacagatt cccaagaagc tactaccgat gcacgcatca aggatgtaac gtgaagaaac 120
aagtacaaag gctgtcaaag gatgaaggag tagtagtaac tacttatgaa ggcatgcatt 180
cacateceat tgagaagtee acagataact ttgageacat tttgaeteag atgeaaatet 240
atgetteett ttgaaaegte cateaettea atgeetaagg catgaeacte aattagteae 300
ttgtaaaata gtactacagt atattgtgta catgcgtttt gaacctagat gctatatttt 360
gaaataaaac gcaacttcat tagggaattt aatttgatca ttgtacaact aaaagtaatg 420
ttgctatttt tttgttttta tcactttgtt tttgccggag ccatgncttc attttaactc 480
tttcttttag aattaacaaa taattncatg ttggagaaga ncgtg
                                                                  525
<210> 118
<211> 225
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g17-3-2
<400> 118
gaccaaatga gcaaattgaa gaaatgctgg agatcaccac atacttccag gcaaagcaac 60
ctcaattttt qttaccaaaa qatttcttga ttaaactttt gaaagtaaac acgtgtgtgt 120
agagaagtaa atgcaggcac tgggatttca atatcgtttc attgatgctg gtacagtagg 180
                                                                  225
agattgaaac taaacatttt cttgaagttc agtacgtgtt cattg
<210> 119
<211> 412
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g18-4-7; homology with L18 (60S)
      ribosomal protein
<400> 119
attgagaagg ctggaggaga atgcttgacc tttgatcagc ttgctcttag agcccctctc 60
gggcagaaca cggtactgct taggggtcca aagaactcgc gggaagctgt taaacacttt 120
qqtagaqctc ctggtqtccc acacagccac acgaagcctt atgttcgggc aaagggaagg 180
aagtttgagc gagcaagagg gaaaagaaag agcagaggtt tcaaggtttg aggaattgcg 240
agtgtttgag tgcacgatga gagaatttct tttagaaggt tttccctacc tacttttac 300
catattagct tcttttctt gtcgaatttc ttatttcacc cctgtttctg tgacactcca 360
acctatagcc gattttgaat gcttttatta tctattctac gaaattaagc tg
                                                                   412
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<210> 120
<211> 373
<212> DNA
<213> Nicotiana tabacum
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<223> plasmid g18-5-1
<220>
<400> 120
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gaaatttgtt gtaatcttca agaatgtact tgttgccatc aatagaaaag caaacaatat 120
tgtgttcagt tacagccttg ttgggtcttg ctgagagtta tttttctagt tcctgaaagt 180
tatcttgcaa gctatcatgt agctgtgtgg taattttcac aggtttgagc tacagttgaa 240
gccagtaaca tgtgttgata ttatagctaa aataactaat gcttacctgc agtttccgtt 300
tgtgtggaat aaggagaaga attgatgtgt aagcatggct tctgtgagtt gactctatta 360
                                                                   373
tctattgcat tac
<210> 121
<211> 390
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g18-5-12; homology with
      capsanthin/capsorubin synthase, promoter region
<400> 121
ggttgcaagg gtgtatccga accetatttg cagaaaaatt atactgtata tacaaggtca 60
aaattattt ttctgtttat atagttagat gttaaattgt cttggctttt tcgtgtattt 120
atttctttat attttgaatc ttcttggtga aaatcctagc tctgtacaca caaagagccg 180
acatgctgat ctctctctc ctctggacgg agagtcttct gaagtgattt tgtgcttctt 240
cagtgtgttt atagatcaat ttagtgtctt tgtcaaatgg atttctaagt gaaaaaagag 300
aaaaagtatt tcaatgcgtg tgacctacct tgcataaact ctgcatgatg gatatacaat 360
                                                                   390
gtttctgctt gatatatgta tatgttttgg
<210> 122
<211> 381
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g18-6-12
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aatttcattt actataattc agatgtatct gtgtacaagg cagccgtgtt attctgtttt 180
gttgaattcg cgcacctgca ttctcctgct gttttttgtt aaatctcttt ctttttcctt 240
cttttgcccc cgttttatgt ctgtttgcgc ggcagggaca gaaacagaga aaccgccgtg 300
taattaagat aaaagctttc agcttattca gaagatcttg aatatgctat aattttaatc 360
                                                                  381
tctcacaaac tgtgtatctt t
<210> 123
<211> 356
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g18-6-5
<400> 123
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tttcaaaaaa ttttagttgt catttctctt ctggtctttc ctttccagtc gatctcttct 120
tcagaaaaca aaaaaaatg gttcaacttt agttttgagt ccagatttga tctcatttct 180
ttgctagagt ttcgtttgct gttatttgct ggttttttgc tttacccgtg gctgaacttc 240
cttcatcttt atttctgctc tctaccagct atttcgagct ttatttgtta agtattctag 300
gtacacactt tcaaatctgt actgtttctt catgaaaagg gctgaaaatt ttgaat
                                                                  356
<210> 124
<211> 293
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g18-7-5 ; Arabidopsis genomic homology
<400> 124
aagaaaagta gcaccagggg cttgtccttg ttgtggagga aaagtacaag ctgtagatgt 60
agaaggccgt ttcagatttt gctttctccc tatttgcttt aggttcaaga ggaagtatct 120
ctgtactctc tgttctaagc gtttggtttt gtattcttga tctccctatt ttcctcttgt 180
aatttctact ctcaattttt tgaacagcat cctataagtg taattattta tttgaaatag 240
tgtttgagag ttgttcattt gctcaagaat atatgaaact tttgtagttg tgc
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<210> 125
<211> 259
<212> DNA
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<213> Nicotiana tabacum

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<220>
<223> plasmid g18-8-7
<400> 125
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cgatgaaaac tagaatgatg tttttttct caagtaaatt tatntcattg tatttcttgt 120
tagtttttct cttctccact cccctctgtt tttctgtggc gcataggttg tacattgtaa 180
aaatttccca ataccaacat aatttaagga tgtaaaccat cttcttgctt tgcttgtaat 240
ttctctacta ggttgcttt
                                                                259
<210> 126
<211> 491
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g19-1-5; Arabidopsis genomic homology
<400> 126
ggttttaata agcttattgg tggttggttg ttcgagtttt ttggttactt taggagaggc 60
aagtggtagg tggacgagtt ttggggttat atttcaaatg gtagtgagtt caggatttgc 120
aactctqtta atqcttcaga gtcttgctgt gaacgtggtg ttgtatatgt attgcaaggc 180
atatcgtggg gagctggcgt ttgagatcgc ggaggagttt gcgagtcagt atgtgtgttt 240
qccttttgat aatqaqaaqq ttcctcatct tqtttqtqtt qttcaaqatt qaatqtgcct 300
aaggtcagtg agattatgtt aggatgatgc agttagtagt ttgaagaagt agtgttttgt 360
tttactcgta gcatgtatat agtttcttgt ttgttagata aatgattgaa gatgtgtgtt 420
acctgttggc aatgtgcatc tttatatgta aaaaaagctt aatacctgtt atgaaattcc 480
                                                                491
ctccnagttt t
<210> 127
<211> 485
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g19-1-6
<400> 127
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ataatttttc tttttctaac caaagcaaaa taatatcatt tgtgaaattc agtcggtgta 120
cctgaacatt attagtatta aaatggagaa atgagagaac acgtatggcc actagagata 180
ttaaaqctac ctaaatatga caatagatga agcagaggac agtataatat aattttcttt 240
ttactataat aatcatctct ctctaggcgg ctagttggga ctatgctcaa cttgcaatat 360
ttaattttgt tttcatgttg ttcctttttc tggatgatgt tttaactgtc gaaaaaattg 420
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agagctaagt tgcatggttc tgagttcgaa ggattaaaag caatgtnaat caattggctc 480
                                                                  485
tatgc
<210> 128
<211> 484
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g19-1-7; Arabidopsis genomic homology
<400> 128
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tcaatgaagg cagaaacagc ggttttgaat ccacctctca tctcttttga caacaagagg 120
gatgcttatg gatttgctgt acgacctcag catgtacaaa gataccgtga atatgctaat 180
atctacaagg aagaagagga agagaggtct gataggtgga acgatttttt ggagcgtcaa 240
gcagagtctg ctcagttacc cataaatggg atatctgcag acaaaagttc tactaatcct 300
ggtgccaaac catttagtca ggaggtaagt tgtgatgcac agaacgggga agaaggtcaa 360
cttgaaaatg caactgagaa ggatgtcata ctgacctctg tggagaggaa aatttgtcag 420
actcagatgt ggacggaaat tagaccctct ctacaggcag ttgaggatat gatgaacact 480
                                                                   484
cgtg
<210> 129
<211> 224
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g19-2-1
<400> 129
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atagagttaa aggccagatc atgtctgcta tgagtcatca tctgttgttg gaagagaatt 120
cacttgttta attttacttc tcatatttta tatcatggga tttcatgttg gatggatgga 180
                                                                  224
ccaqtqtqta tqtcaaatta attcttattq cgaaaaaaaa aaaa
<210> 130
<211> 198
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid g19-2-9
<400> 130
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aaattctgta aagtatgcac atctgggtga ttgattgttg catacatgct aatttatcag 240
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tggttggttc gattg
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<223> plasmid g3-1-1; Arabidopsis genomic homology
<400> 135
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qaaaqcatqt tcaaqtctat gcattatgaa atccgtatga cctattcaaa ggacaaggaa 120
ggattgttgt gtgtgcagaa aacaatgtgg cgaccaacgg aggttgagac actaactaat 180
gcccttgctt agctgcttag cgtgtgtgcg gatgctggtt gtatatcatt cgagaggctt 240
tcatgccacg gtgactagat agtttttcga ttaaattctt gttactgtat tcttgtcagg 300
ctaccgtgta ccattccata gcaaaattag tgctattatc actatatatt tgtggaaagt 360
aagttttqta atattatgtc attagttgtg gaggaggtgg acattcttgg aattgtaaat 420
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      factor
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caatgaatgc tgctgaaata actgataagc ttggactcca ctctctcagg cagcgtcact 180
ggtacatcca gagcacttgt gcaacttctg gagagggact ttatgagggg cttgattggc 240
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gatgcaggcg ggtttttatc tagttctttt tccttttttt cccgaacatt cccagaatct 360
gtgtggttat gaatateeet tgaaagtgat ttgettettg gtaggaceta ttgaaatgtt 420
tttgtaatac agtggttgga tatatgtaat tgtttgttta gtttaaagta taatgctata 480
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tgtttgttcc ttc
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<211> 501
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<223> plasmid g6-2-13; homology with ACC oxidase
<400> 137
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tgaaccctaa gcctagtgat gtaattggtc ctttggcaga agtgctagag aatggagagg 180
aaccaattta caaacaagtt ctttactcag attatgtcaa gcatttcttt aggaaagctc 240
atgatgggaa agacactgtt gattttgcta aaatcaagta gaaattagtg gatctgctcg 300
aagaataaga agtgcgctta tattaagcta atgtattttt ctttcatgta tttttagtta 360
cgactactca gcaatttaaa aaaaaagaag agatagtctc atactgcaaa gtataggaga 420
atatttttgg gattaattag gtgttcgaat tttgtaccgg ataaattata attgagctgc 480
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tgatattatg gcaaatttag c
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<220>
<223> plasmid g6-3-7; homology with ATP citrate lyase
<400> 138
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atgttctcta caccaagtga agacgctccc aatagcagca cgcagaaagt cgcctgcttc 180
ctatccagca ttttatcgaa aagtgtttgt ttagtcattt gttgtgatca ttcttcttgt 240
tttctgctag tattttgtac tcctaagaac ttgctaagca tttctgtaag ttgttataag 300
agacaactet tttagtttea caccaagagt tteetteaat teetatatat caaagaaata 360
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<223> plasmid g6-4-4
<400> 139
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attettettt egteeaaaca ttaagttaaa taagttaeta eateatttaa tetteettaa 180
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tgcattgtca cagtgtaagg ttggaagcaa ataatatatc ctgcttaatg tcgtttggtc 300
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<223> plasmid g6-4-5
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accatcacta actagttctc catttttctt actggtgtat ttactttcaa gtattttatt 180
tgatgaggcg atatctcatt acttttgttt ttccagttgt ttgctttagt gaatttatat 240
gctggaagga tttgaggtat tagatagaaa gcatcttctg atttaacttc aattatgtg 299
<210> 141
<211> 356
<212> DNA
<213> Nicotiana tabacum
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<223> plasmid g7-1-1; homology with a A. thaliana gene homologous to MEI2 (meiotic regulator)

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C

481

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<211> 480
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<213> Nicotiana tabacum
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<223> plasmid g9-2-6
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cttcttacgt cccttttata cctgtactat aagtaggtag gtggtggcct gaaatcccat 180
aagccaaaaa aaatatacaa gtaagcttca ccatgctcca ttacttagaa actgtacagc 240
ttgtgattta ccaaatatgt ctacattagt cctaatattt ccttagatat acgtagccta 300
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tgttgactaa cttctcagca gttgcaagtg aatttcattt attgtttgct attttcctgc 420
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<211> 447
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<223> plasmid g9-3-17
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<223> plasmid g9-3-4
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 gcaaggtggc tctggccgaa taaatgccaa ttatgattga ttgatgagga ggctaaaatg 180
 tggatttagg tctttttagt ttgtgatgga tagcaaactt accggataat ctttgcttag 240
 tetgeatgte tggtggtgea gtettaggtg gtagettttg acgtggtaaa agagaatttg 300
 ttggccaatg tcacacgggt gagctggact acagccgggt tttgccacat ggttttggga 360
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 agaacattaa aatagttgcc cattttctcc
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 aagctatttt agtccaattt tgacttaatt gaggaatatt ataattaagc tatgttagtt 180
 caattttgaa cttaattagt tctttcatta ttccttgttg ggctgtaatt tgacatttct 240
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 <211> 245
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 <213> Nicotiana tabacum
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 <223> plasmid g9-6-1; homology with LOX lipoxygenase
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 ttaagcagga gtggcaacag atgtgtgtag atctattttt atgtcaatat ttgtttagcc 180
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<223> plasmid t12-1-7
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gccttttcat gttttgcctt tatgtttttc aagctgaaga acctgcacat ttgcagaatc 300
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                                                               353
<210> 150
<211> 351
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid t12-2-1; homology with chitinase class 4
<400> 150
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ctcanaacat atatatgtcg ncctcactac cgggggagca actaatantg aatatctnng 120
qttatncttt qattcaactn ctqqnnatna cttacqtcct aacatqtnaq attatcccca 180
gtetecagae ecagtngttg acganactea gtataataet cageeetten ggeaacagte 240
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accttgggna naagntgatg aaagaatggg ngnttggtnc gnncgatanc a
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<211> 352
<212> DNA
<213> Nicotiana tabacum
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<223> plasmid t12-2-18
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ctggaagccc ttcattgatg tggagtgtaa acgtggtnct ataagttant tctttcgtgt 180
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caataaanga gatcttngcc taaacanatt cgnggacnag cgtgaaatgn tagggaatgt 300
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<213> Nicotiana tabacum
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<223> plasmid t18-2-5 ; homology with basic PRB-1b [I]
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ttccaactga tgtctagtaa taacggttta cgtgatcaaa taatgaataa aagctttgtc 180
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cagtggataa taatccaatg gtgtagcaag gggtggattt actgttatct acttgtttta 300
catttgtttt tggtggtatt atggaggtgt gtatatgtat gtgttttgat gaataaacaa 360
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<223> plasmid t18-3-2
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cattetttea atgtgtgeag ttacatecet atettttggg aggatacate atectegnea 180
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<211> 366
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid t18-3-6; homology with chloroplast RNA
     binding protein
<400> 154
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tagagaacaa ttggagaatg cccttcaaaa tcttaatgga gtggaactgg atggaagggc 120
aatgcgcatt agcttagcac aagggaagaa acaataagat ggacaagatt cttgtatatt 180
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aatttctagt cqcataqctt cataataatt ctgcaaagct tccgcgctaa tttccttcgg 180
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ggaattactc gtaatangat attggctaca attgaaaagg tettateaat aaaattteca 300
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<211> 184
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<223> plasmid t2-6-3
<400> 158
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                                                                  184
gccc
<210> 159
<211> 534
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid t7-1-12; homology with SNF-1like kinase,
      calcineurin B-like calcium sensors interacting
      protein in Arabidopsis
<400> 159
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gatttacgag gtggcacctt cactatacat ggttgcttcg caaggctgga ggagatacct 120
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cgagtttata atacattgtt ttatgtacga ttaaggcacg taaacttaga aaaattaaga 420
ctggttttac attgccattg ttgtcttatt tggtgacaag atattacgga tcaatacccc 480
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<210> 160

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<211> 398
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid t7-2-4 ; homology with a multi-functional
      protein -beta oxidation
<400> 160
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tttggagcag agagctgcaa aaggattgcc cttgggagga tcgtgttgag ctgcatatca 180
tatgatcata tccttgcaga agaagcagta attcaagcat gctgaacttg tgntcggaaa 240
taaggcgggn aagtttgtta attacaatta gttagnagtt ccattaatta taataatttc 300
ctattttttc ccctcaagtt atttgatggt agttgtaact ttggctctac aaantagtgt 360
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<210> 161
<211> 398
<212> DNA
<213> Nicotiana tabacum
<220>
<223> plasmid t7-4-7; homology with GST (bronze-2
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35 40 45

Leu Met Ala Ser Met Glu Ala Pro Ser Ser Ser Val Val Thr Asp His
50 55 60

Pro Asn Ser Ile Pro Tyr Asn Pro Asn Asp Gln Asn Glu Val Arg Ser
65 70 75 80

Gly Lys Lys Asn Lys Val Glu Lys Lys Ile Lys Lys Pro Arg Tyr Ala 85 90 95

Phe Gln Thr Arg Ser Gln Val Asp Ile Leu Asp Asp Gly Tyr Arg Trp
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Tyr Tyr Arg Cys Thr His Gln Gly Cys Asn Val Lys Lys Gln Val Gln 130 135 140

Arg Leu Ser Lys Asp Glu Gly Val Val Val Thr Thr Tyr Glu Gly Met 145 150 155 160

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